The widths of the spores, taken at the widest place, were approximately 2μ. Slight variations occurred in most cultures, the spores being, if anything, slightly wider than those from the field.

TABLE 2.

Selenophoma donacis *var.* stomaticola (*Bäuml.*) *Sprague and A. G. Johnson.*

S.U. Acc.	Date.	Collector.	Host.	Locality.	Pycnidia.	Pycnidiospores.	
						F.	C.
234	30. 8.50	D.S.	*Stipa aristiglumis.*	Piallaway, N.S.W.	—	12–20	12–20
456	29. 1.51	D.S.	*Phleum pratense.*	Kosciusko, N.S.W.	40–100	10–14	12–16
466	29. 1.51	D.S.	"*Poa caespitosa.*"	Kosciusko, N.S.W.	60– 90	12–16	—
467	29. 1.51	D.S.	*Agropyron scabrum.*	Kosciusko, N.S.W.	80–100	14–20	—
469	1. 2.51	D.S.	*Danthonia penicillata.*	Kosciusko, N.S.W.	50– 70	12–16	8–14
473	7. 2.51	G. Wade.	"*Poa caespitosa.*"	Cressy, Tas.	60– 80	16–18	—
497	20. 3.51	T. W. Atkinson.	*Festuca elatior.*	Glen Innes, N.S.W.	—	12–20	—
517	2. 3.47	A. B. Costin.	"*Poa caespitosa.*"	Kosciusko, N.S.W.	50– 90	10–20	—
532	7. 7.51	I. A. Watson.	*Stipa* sp.	Tichborne, N.S.W.	70– 90	10–14	12–16
595	17. 9.51	A. T. Pugsley.	*Triticum vulgare*, "Scimitar".	Adelaide, S.A.	—	12–18	9–16
626	17.10.51	I. A. Watson.	*Amphibromus Neesii.*	Tichborne, N.S.W.	50– 90	10–16	10–16
635	22.10.51	D.S.	*Vulpia Myuros.*	Temora, N.S.W.	60– 80	8–12	10–16
637	22.10.51	D.S.	*Danthonia caespitosa.*	Temora, N.S.W.	50– 80	12–18	10–18
651	11.12.51	J. Begg.	*Dactylis glomerata.*	Canberra, A.C.T.	60–100	12–16	10–20
670	19.11.51	E. J. Breakwell.	"*Poa caespitosa.*"	Canberra, A.C.T.	60–120	12–14	12–16
681	23.10.51	P. G. Valder.	*Stipa aristiglumis.*	Gunnedah, N.S.W.	—	14–20	—
696	27.12.51	D.S.	*Anisopogon avenaceus.*	Oatley Park, N.S.W.	50– 90	10–16	10–16
705	23. 1.52	D.S.	"*Poa caespitosa.*"	Mt. Ainslie, A.C.T.	60– 90	12–16	10–20
706	23. 1.52	D.S.	*Agropyron scabrum.*	Mt. Ainslie, A.C.T.	80–100	12–16	—
709	23. 1.52	D.S.	*Danthonia* sp.	Mt. Ainslie, A.C.T.	50–80	12–16	10–16
710	23. 1.52	D.S.	*Amphibromus Neesii.*	Sullivan's Ck., A.C.T.	60– 90	12–16	14–20
711	15. 6.48	R. A. Perry.	*Neurachne Muelleri.*	Gallipoli Station, N.Territ.	60– 90	10–14	—
733	27. 4.52	D.S.	*Phleum pratense.*	Kosciusko, N.S.W.	80–120	12–16	12–18
756	8. 6.52	D.S.	*Aristida vagans.*	National Park, N.S.W.	—	—	14–16
776	12. 8.52	D.S.	*Aristida vagans.*	Mary's Mount, N.S.W.	100–120	14–16	—
780	16. 8.52	D.S.	*Agropyron* - wheat hybrid.	Botanic Gardens, N.S.W.	75–120	10–14	10–18
787	26. 8.52	E. G. Wingrave.	*Dactylis glomerata.*	Huonville, Tas.	—	—	10–18
799	16. 9.52	F. Robertson.	*Microlaena stipoides.*	Sublime Point, N.S.W.	—	—	10–14
802	16. 9.52	F. Robertson.	*Dichelachne rara.*	Sublime Point, N.S.W.	70–100	10–16	10–16
819	24.10.52	D.S.	*Danthonia caespitosa.*	Temora, N.S.W.	60– 80	12–16	13–18
823	24.10.52	D.S.	*Amphibromus Neesii.*	Temora, N.S.W.	40– 90	14–16	10–16
826	24.10.52	D.S.	*Stipa variabilis.*	Temora, N.S.W.	60– 90	12–18	—
838	31.10.52	D.S.	*Anisopogon avenaceus.*	Kellyville, N.S.W.	60– 80	10–16	—
851	14.11.52	D.S.	*Microlaena stipoides.*	Mt. Tomah, N.S.W.	60–100	10–14	10–17
861	12.11.52	D.S.	*Dactylis glomerata.*	Orange, N.S.W.	70– 90	12–14	10–20
866	18.11.52	G. Sullivan.	*Microlaena stipoides.*	Bilpin, N.S.W.	75–100	10–14	10–20
867	18.11.52	G. Sullivan.	*Danthonia racemosa.*	Meadow Flat, N.S.W.	50– 90	14	—
874	8.12.52	G. Sullivan.	*Amphibromus Neesii.*	Sullivan's Ck., A.C.T.	50– 60	12–16	16–20
875	8.12.52	G. Sullivan.	*Agropyron scabrum.*	Mt. Ainslie, A.C.T.	60– 80	14–18	10–20
876	8.12.52	G. Sullivan.	"*Poa caespitosa.*"	Mt. Ainslie, A.C.T.	50– 60	12–16	10–15
877	8.12.52	G. Sullivan.	*Deyeuxia monticola* var. *valida.*	Mt. Ainslie, A.C.T.	50–100	14–20	16–23
884	17.12.52	G. Sullivan.	*Danthonia pallida.*	Gunning, N.S.W.	50– 95	13–20	14–20
894	4. 3.53	D.S.	*Sporobolus capensis.*	Cronulla, N.S.W.	50– 95	—	14–18
904	15. 5.53	D.S.	*Sporobolus elongatus.*	Camden Park, N.S.W.	50– 70	12–15	12–16

Spores were uninucleate (Plate ix, 4), or binucleate in dividing spores produced in culture (Shaw, 1953).

Cultures from all isolates were at first mucous, pale cream to faint pink, and produced masses of conidia directly on the mycelium. Old cultures became leathery or carbonaceous, and the texture varied considerably. They were variously coloured, but were mainly black with yellow or pink, with or without yellow or rose pigmentation of the medium. The colour of the colonies and the intensity of the medium pigmentation varied with the age of the colony and the amount of exposure to light.

Cultures were mainly of three types:

1. Coloured yellow or yellow and black, with bright yellow pigmentation of the medium. (Isolates from *Amphibromus Neesii* and *Agropyron scabrum*. The isolate from *Dactylis glomerata* was also of this type, but it had been isolated from old faded leaves.)

2. Cultures black, pink, or black and pink, with rosaceous pigmentation of the medium. (Isolates from *Triticum vulgare*, *Phleum pratense*, *Microlaena stipoides* and *Agropyron*-wheat hybrids.)

3. Cultures without medium pigmentation under the conditions during which the tests were carried out.

Isolate from Wheat.

In September, 1951, leaves of "Scimitar" wheat received from the Waite Institute, South Australia, were infected with *Septoria tritici*, and also with a few lesions of the eyespot type which were not typical of the speckled leaf blight. Upon examination it was found that the lesions were caused by a *Selenophoma* with small spores. Pure cultures of the organism were established.

As mentioned previously, the *Selenophoma* from wheat in the United States was first reported as the var. *stomaticola*, but was later placed in the species proper, mainly because of the shape of the spores which were reported as being 16–21 × 2·2–3·5μ (Sprague, 1950).

The spores in the collection from South Australia measured 12–18 × 2μ, and from culture measured 9–16 × 2μ. When produced on "Rhodesian" wheat in the glasshouse they measured 10–18 × 2–2·5μ. The measurements, which are all in the same range, are slightly smaller than those given by Sprague, but his drawings of spores on leaf fragments from Australia which were intercepted by Pollock, and of spores from Idaho (1950, his fig. 22, B and C) are very similar to those of the present collection, except that the latter are slightly narrower and slightly more pointed. The organism resembled the small spored variety on grasses rather than the large spored species on *Arundo donax*. While it is realized that the wheat *Selenophoma* in the U.S.A. was transferred to the species proper only after much consideration, it is felt that the South Australian organism is more accurately assigned to the var. *stomaticola* than to the species (Plate ix, 5–10).

Sprague (1949, 1950), in inoculation tests with the American organism, could obtain only sterile leaf spots. When inoculated in the glasshouse, the Australian isolate produced on wheat, leaf spots with abundant pycnidia and spores. It is, perhaps, a more virulent strain. The pycnidia produced after artificial inoculation, however, were not heavily pigmented and could not be discerned with the naked eye.

Inoculations were carried out on the following plants in several series of tests, using spores from culture: *Avena sterilis algeriensis* "Algerian", *Bromus inermis*, *Dactylis glomerata*, *Festuca elatior*, *Holcus lanatus*, *Hordeum distichon* "Kinver", *Phleum pratense*, "*Poa caespitosa*", *Secale cereale*, *Triticum vulgare* "Federation".

In every test, lesions with pycnidia and spores occurred on "Federation" wheat, but no infection could be obtained on the other grasses or cereals. This confirms Sprague's finding that the wheat isolate is confined to wheat.

Various species of wheat and those varieties either agronomically popular in Australia or being used as sources of resistance to other diseases, e.g., leaf and stem rust, were tested in the glasshouse for their reaction to the *Selenophoma* isolate.

Sprague (1949) noted that resistance varied in the field from the highly susceptible varieties "Rex" and "Orfed" to the highly resistant varieties "Kharkof", "Comanche" and "Hymar × Elgin 3 (F4 composite)".

Wheats tested were divided into the following categories:

Resistant.	Mod. Resistant.	Mod. Susceptible.	Susceptible.	Very Susceptible.
Triticum monococcum.	*T. timococcum.*	*T. vulgare;*	*T. compactum;*	*T. dicoccum;*
T. monococcum var. *flavescens.*	*T. vulgare;*	Brolga.	Little Club.	Khapli.
	Democrat.	Celebration.	*T. vulgare;*	*T. vulgare;*
	Exchange.	Charter.	Bordan.	Dundee.
	Hofed.	Chinese × (Chinese × *Agropyron elongatum*).	Egypt 122S.	Festival.
	M.D. 1303.	Eureka.	Federation.	Kenya 744.
	Mentana.	Fedweb.	Resistant.	*Rhodesian.
		Gular.	Bencubbin.	Yalta.
		Kendee.		
		Marquillo.		
		Mediterranean.		
		R.A.C. 170.		
		Thew.		
		Timopheevi der. 1656.		
		*Uruguay 1064.		

* " Rhodesian " and " Uruguay " are shown in Plate ix, 2 and 3.

Organisms from Various Hosts.

The results of pathogenicity tests which it has been possible to make to date are as follows:

	Agropyron— Wheat hyb.	*Aristida vagans.*	*Dactylis glomerata.*	*Phleum pratense.*	*Triticum vulgare.*
Agropyron scabrum				—	
Amphibromus Neesii			—		
Aristida vagans		S			
Arrhenatherum elatius	—	—	—	—	—
Avena sterilis algeriensis	—	—	—	—	—
Bromus inermis				—	—
Dactylis glomerata	—	—	S	—	—
Danthonia caespitosa	—		—	—	
Dichelachne sciurea				—	
Festuca elatior	—	—	—	—	—
Holcus lanatus	—	—	—	—	—
Hordeum distichon	—	—	—	—	—
Phleum pratense	—	—	—	S	—
"Poa caespitosa"	—	—	—	—	—
Poa compressa	—		—	—	
Secale cereale	—	—	—	—	—
Triticum vulgare	L	—	—	—	S
Vulpia Myuros				—	

S = Susceptible; L = Lesions only; — = Immune.

All the grasses inoculated are recorded hosts of *Selenophoma* here or overseas. Each isolate tested was specific for its own host. Infection was easily obtained on timothy and wheat with the respective isolates, but difficult to obtain with the other isolates even on the hosts from which they were obtained. It is possible that the conditions favouring infection were not present or that there were genetic differences in clones of the same grass species. Tsiang (1944) found highly significant differences in reaction to *Selenophoma bromigena* between clones of *Bromus inermis*.

Economic Importance.

Selenophoma donacis and *S. donacis* var. *stomaticola* have been identified on both native and introduced grasses throughout the eastern half of Australia. It is not known how long the organisms have been present in this country: whether they are indigenous on the native grasses or whether they were imported here from overseas on introduced grasses and have since spread to the native species.

Some of the grasses collected were heavily diseased, but, as the cross-inoculation tests to date indicate a great deal of specificity for the host, it is probable that the disease will be serious only on particular species in certain localities.

The first world record of a *Selenophoma* on wheat was on Australian wheat examined at Quarantine Station at California in 1944. It was not recorded elsewhere until 1948, at Pullman, Washington, but Sprague has since stated that it had been collected at Pullman in 1915, but not then determined (1950).

The only known field occurrence in Australia is that on "Scimitar" leaves from South Australia (S.U. Acc. 595). However, it is instructive to examine the proportions of the well-known pathogenic fungi in the five shiploads of wheat identified at the Californian Quarantine Station (Pollock, 1945), viz.:

Fungus.	Number of Lots.
Urocystis tritici	7
Puccinia rubigo-vera var. *tritici*	17
Puccinia graminis var. *tritici*	5
Tilletia caries	1
Tilletia foetida	1
Selenophoma sp.	5
Septoria tritici	17

The writer has not been able to determine the season in which this wheat was grown or from what parts of the wheat belt it came. It is evident, however, that the disease was present in the field somewhere prior to 1944.

It should be noted that many of the varieties of wheat which are grown commercially in Australia, or used as sources of resistance to other diseases, proved susceptible to the South Australian isolate in glasshouse tests. Thus the varietal composition of the wheat belt makes it a suitable medium for the organism, although environmental conditions in the field might be operating against high incidence and widespread distribution.

Acknowledgements.

Grateful thanks are extended to Professor W. L. Waterhouse for his unfailing interest and advice, to Miss J. W. Vickery for identifying many of the grasses, to Mr. S. Woodward-Smith for some of the photographs, and to Mr. C. S. Christian, of the C.S.I.R.O., for permission to examine the grass collections of the Northern Territory Land Regional Survey for diseases. The help of all those collectors who forwarded specimens from New South Wales and the other States is also gratefully acknowledged.

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DESCRIPTION OF PLATE IX.

1. *Phleum pratense* with "frog-eye" type lesions caused by *Selenophoma donacis* var. *stomaticola* after artificial inoculation. × 1.

2. "Rhodesian" wheat, a very susceptible variety, with lesions and yellowing caused by *S. donacis* var. *stomaticola* after artificial inoculation. × 1.

3. "Uruguay" wheat, a moderately susceptible variety, with lesions caused by *S. donacis* var. *stomaticola* after artificial inoculation. × 1.

4. Spores of *S. donacis* var. *stomaticola* from *Sporobolus elongatus* stained with Giemsa to show one nucleus per cell. × 900.

5. Spores from culture of *S. donacis* from *Arundo donax*, stained with cotton-blue. × 600.

6. Spores from field collection of *S. donacis* from *Arundo donax*, stained with cotton-blue. × 600.

7. Spores from culture of *S. donacis* var. *stomaticola* from wheat, stained with cotton-blue. × 600.

8. Spores from glasshouse collection of *S. donacis* var. *stomaticola* from wheat, stained with cotton-blue. × 600.

9. Spores from culture of *S. donacis* var. *stomaticola* from *Phleum pratense*. × 600.

10. Spores from field collection of *S. donacis* var. *stomaticola* from *Phleum pratense*. × 600.

Photos 4-10 by Woodward-Smith.

STUDY OF SOIL ALGAE.

II. The Variation of the Algal Population in Sandy Soils.

By Y. T. Tchan, Macleay Bacteriologist to the Society, and Jill A. Whitehouse, Teaching Fellow in Microbiology, Sydney University.

(Plate x, figs. 1, 2; four Text-figures.)

[Read 26th August, 1953.]

Introduction.

The algal population in the soil has previously been studied mainly from a floristic point of view, but little information is available concerning the number and variation of algae in the soil, which is partly due to the lack of an adequate rapid technique. This difficulty has been overcome by fluorescent microscopy introduced by one of us (Tchan, 1952) and, using this new technique, the population of algae in the soil was studied in its natural conditions, and experiments were carried out to explain some of the direct observations obtained in studying the daily variation and vertical distribution of the algae in soil.

I. Technique.

The technique has been fully described in a previous paper (Tchan, 1952) and no modification is introduced in the present work.

The sensitivity of the method may be tested as follows.

A counting chamber of 0·2 mm. depth and a surface area of 1·5 × 1·65 cm. has a volume of 0·05 c.c. Since the soil suspension can be concentrated by centrifuging to 1:5 (soil:water), then this volume represents 0·01 g. of soil. It is found with suitable replicates that the technique can estimate an algal population of the order of 1,000 cells per gramme. A reasonable estimation of a population of about 100 cells per gramme can be obtained by using McCrady's statistical table (Calmette *et al.*, 1948). In fact, estimations using the statistical table allow for a theoretical possibility of a population as low as 20 cells per gramme of soil. In practice, little interest or significance is attached to such a low algal population in the soil. The technique used is described as follows.

A suspension of algal cells was counted over the whole chamber. A series of dilutions (1 c.c. in 9 c.c. of water) was used (five replicate counts for each dilution) until the last dilution was free of algae, e.g., one suspension contained 80 cells per chamber. In the first dilution the replicates gave five positive countings; the second dilution gave three; and the third dilution was free of algae. According to the statistical table the above results give characteristic numbers of 553 or 530, which correspond respectively to 90 and 80 cells calculated to be present in the original suspension. This gives a good correlation with the initial count made on the undiluted suspension.

In another suspension which gave a theoretical number of 500 cells, the results were as follows:

Characteristic number	520	513	511	451	503
∴ Cells in suspension (calculated from table)	500	850	450	500	600

The use of the statistical technique was found to be necessary when the algal population was at a very low level. For higher numbers the direct count of the suspension was quite adequate.

In order to compare this direct microscopy technique with a culture method the following experiments were carried out.

A sandy soil was used to prepare a soil-water medium according to Pringsheim (1950). Potassium nitrate and potassium phosphate buffer adjusted to the same pH as that of the original soil were added to the medium.

The algal population in a suspension was first estimated by the direct microscopy technique. A series of dilutions of this original suspension was then inoculated into the soil-water medium, using five replicate tubes for each dilution prepared. The number of algae present after incubating at 25°C. with two fluorescent lamps for several weeks to three months, was calculated by using McCrady's table, and depended on the number of tubes in each dilution in which growth was evident when examined either by naked eye or microscopically. The results are summarized as follows.

Number obtained by direct microscopy	1,000	1,400	2,000	4,000	23,000	29,000	43,000	40,000
Number obtained by culture technique	600	1,500	1,500	4,500	13,000	30,000	30,000	45,000
Ratio $\frac{\text{culture}}{\text{microscopy}}$	0·6	1·07	0·75	1·12	0·56	1·03	0·70	1·12
Culture technique shows:								
Less	40%		25%		44%		30%	
More		7%		12%		3%		12%

The above table shows that significant differences between the two techniques occurred when the culture technique gave a lower number than obtained by direct microscopy. When the culture technique gave a higher number, the difference was only of the order of 12%. The rapidity of the direct microscopy is very appreciable, and for this reason the culture technique was not used in any of the other experiments carried out, except on rare occasions.

II. Daily Variations in the Algal Population of the Soil.

For the following experiments described a garden soil from Sydney University was used. The soil was apparently homogeneous as a result of previous cultivation. Samples were prepared by mixing five small amounts of soil taken at random from within a square metre.

The experiment was set up at 11 a.m. in May on a sunny day. The following variations in the algal population were recorded at intervals during the day:

Time	11 a.m.	2 p.m.	5 p.m.	7 p.m.	8 a.m.	12 noon
No. algae per gramme	4,750	2,100	5,300	4,500	1,300	2,500

Several factors account for these variations. Using the direct microscopy technique it was evident that some nematodes, displaying a white-green fluorescence, had eaten several algae, as seen by the red fluorescent areas within their bodies. Likewise, several protozoa contained algal inclusions. The number of nematodes and protozoa, however, was not high enough to have much effect on the number of algae.

Other factors must be taken into consideration. In winter, water condenses on the surface of the soil during the night; on the following morning the temperature of the surrounding soil rises with the increase in sunlight. Algae thus have a suitable condition in which to multiply; then during the day the soil may dry out, resulting in the death of some of the algae. To support this hypothesis the following experiments were carried out.

Firstly, an experiment was set up to determine the minimum water content of the soil in which the multiplication of algae was possible.

A sandy soil sample was air dried and five grammes placed in the lid of a Petri dish. Sufficient water was added to bring the moisture content of the soil to 12, 24, 30, 45, 60 and 100 per cent. of its water-holding capacity. The lids of the dishes were then covered by the base of the Petri dish so that the bottom of the dish rested on the soil in the lid. The space between the Petri dish and lid was thus reduced to a

minimum. The apparatus was sealed to prevent the loss of water during incubation, and the Petri dishes were incubated at 25°C. for a few hours with two fluorescent tubular lamps, and then the algal cells were counted.

Examination showed that there was no growth of algae below 12% of the water-holding capacity of the soil. From 24% to 30% the number rose from 2,500 per gramme to 4,200 per gramme. At 45% the number was 4,700, and from 60% to 100% the number of algae was approximately constant at 6,000.

The following points are evident from the foregoing results, and apply at least to the sandy soil used in the experiment.

(1) When the soil is moistened to 60% of its water-holding capacity the optimum condition is reached for the growth of algae. Further addition of water does not increase their growth over the short period of our experiment.

(2) When the soil nears its air-dried condition the number of algae in the soil becomes constant.

(3) The minimum moisture needed for the growth of algae is between 12% and 24% of the water-holding capacity, which is indeed very low.

The object of the second experiment was to determine the effect of drought on the algal population.

A sandy garden soil rich in algae was used. Five replicates of 10 grammes of soil were placed in a Petri dish and allowed to dry in the open air and light. Every two hours samples were taken to make estimations of the algal population and the loss of water from the soil. The algal population was estimated by both direct microscopy and a culture technique using soil-water media described above. The loss of water was determined by change in weight of the soil sample. Graph I shows the correlation between the loss of water and the variation of the algal population.

The experiment was set up with soil moistened to 100% of its water-holding capacity. After two hours the number of algae rose from 23,000 to 44,000 (direct microscopy) or 12,500 to 30,000 (culture technique), and the soil moisture content had dropped by 58%. After four hours the count had dropped and risen respectively in the two techniques to 40,000 and 45,000, whilst the moisture content was as low as 12·3% of its original water-holding capacity. From this time both techniques showed a drop in the number of algae (28,000 by direct microscopy and 30,000 by culture technique) and the soil was practically air dried. After three days the soil contained a practically constant number of algae.

Two points are clear from these results: (1) At low moisture levels the growth of algae was not inhibited, but when the soil was almost air-dried (below 12% of its water-holding capacity) the number of algae diminished very quickly; (2) When conditions were suitable algal populations could be doubled in a few hours.

From these observations it is possible to assume that, at least for the sandy soil in question, the daily variation in the algal population is affected by the change in the moisture content of the soil. There is a critical quantity of water which controls the algal population in the soil. This was found to be 12% of the water-holding capacity of the sandy soil used. Below this level no growth could be detected and some algae may have died. Above this level growth recommenced.

III. The Vertical Distribution of Algae in the Soil.

For these observations soils from Kuring-gai Chase Reserve (Mount Colah, N.S.W.) and from Warrah Fauna and Flora Sanctuary (near Woy Woy, N.S.W.) were used. These sandy soils have never been subjected to agricultural treatment or interference.

During the winter and early spring of 1952 soils were sampled from different depths. Precautions were taken to avoid the possible mixture of surface soil with the subsoil. A block of soil was cut out and test tubes pushed horizontally into the block from different levels. On extracting the tube only the portions of the soil near the opening

of the tube were used. This corresponded with the central portion of the soil in the block. The chance of mixing the soil was thus reduced to a minimum. If the soil was water-saturated, the block of soil was cut into slices, which were separated and suspended in water in order to count the algae present.

It is clear that in the water-saturated condition most of the algal population was confined to the top few millimetres. The number dropped very quickly and at a depth of 1 cm. it became insignificant compared with the large surface population.

In the soil which was not water saturated (Mount Colah) the surface soil contained more algae than the lower layers, but the difference was not so sharp. Also a reasonable quantity of algae could be found in a relatively deep part of the soil.

	0–0·4 cm.	0·4–0·6 cm.	0·6–0·8 cm.	1 cm.	1–3 cm.	3–5 cm.	5–10 cm.
Mt. Colah after rain	—	—	—	1,200	900	300	<150
Woy Woy I	800,000	10,000	5,000	<150	—	—	—
Woy Woy II	275,000	15,000	4,000	<150	—	—	—

Woy Woy I—water logged, with macroscopic growth of algae on surface.

Woy Woy II—water saturated, macroscopic growth of algae on surface.

Several questions arise from these observations: (1) Why is the algal population confined to the top layers of the soil when the soil is water-saturated? (2) Is light necessary for the growth of algae in water-saturated sandy soils, as indicated by the presence of algae in relatively larger numbers in the surface soils? (3) If light is necessary for the growth of algae in sandy soils, how far is it able to penetrate into the soils? (4) It is well known that algal growth occurs in the dark if a suitable energy source is provided (Bristol Roach, 1927, 1928). If this is so, can they grow anaerobically in a water-saturated soil?

Several experiments were set up in order to obtain information concerning these questions.

The first experiment aimed to determine the aerobic and anaerobic states in water-saturated sandy soil; the vertical distribution of algae under the experimental conditions; and the effect of light on their distribution. Use was made of the filter paper technique introduced by one of us (Tchan, 1945), in which dyes were used as rH_2 indicators. This technique was successfully used for the study of the aerobic-anaerobic relationship in the decomposition of cellulose in the soil (Pochon and Tchan, 1947).

Pieces of filter paper 4″ × 6″, which had been previously stained in vertical strips with four dyes of different rH_2 values, were moistened and pressed flat against the sides of seven one-litre beakers, so that the colour change of the dyes could be seen during the course of the experiment. The range of rH_2 values given by the different dyes is as follows: methylene blue $rH_2 = 14$, Nile blue $rH_2 = 9$, pheno-safranin $rH_2 = 5{\cdot}8$, neutral red $rH_2 = 3{\cdot}8$. A washed river-sand practically free of algal cells was added in a wet state to the beakers and shaken down well as it was added, in order to avoid air bubbles to a certain extent. The control was set up with tap water. To the second sample Derx's mineral solution was added (Derx, 1950) containing KNO_3 as a nitrogen source. The third sample contained Derx's mineral solution plus 1% glucose as organic matter. The fourth was a duplicate of the second and the fifth was a duplicate of the third, but the beakers were wrapped with black paper so that the light could only penetrate from the surface (surface light). The sixth and seventh were duplicates of the second and third respectively, except that they were kept entirely in the dark. All beakers were kept in a glasshouse.

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